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*J DENT RES* 2010 89: 1219 originally published online 21 September 2010

DOI: 10.1177/0022034510376070

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*J Dent Res* 89(11):1219-1223, 2010

## ABSTRACT

Municipal water fluoridation has notably reduced the incidence of dental caries and is widely considered a public health success. However, ingested fluoride is sequestered into bone, as well as teeth, and data on the long-term effect of exposure to these very low doses of fluoride remain inconclusive. Epidemiological studies suggest that effects of fluoride on bone are minimal. We hypothesized that the direct measurement of bone tissue from individuals residing in municipalities with and without fluoridated water would reveal a relationship between fluoride content and structural or mechanical properties of bone. However, consonant with the epidemiological data, only a weak relationship among fluoride exposure, accumulated fluoride, and the physical characteristics of bone was observed. Analysis of our data suggests that the variability in heterogeneous urban populations may be too high for the effects, if any, of low-level fluoride administration on skeletal tissue to be discerned.

**KEY WORDS:** bone, fluoride, biomechanics, mineralization, public health.

DOI: 10.1177/0022034510376070

Received April 20, 2009; Last revision May 13, 2010;  
Accepted May 19, 2010

A supplemental appendix to this article is published electronically only at <http://jdr.sagepub.com/supplemental>.

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# The Long-term Effects of Water Fluoridation on the Human Skeleton

## INTRODUCTION

The reduction of dental caries *via* the fluoridation of water supplies at 1 ppm is widely considered to be a public health success (McDonagh *et al.*, 2000). However, ingested fluoride is also incorporated into bone during bone formation and remodeling (Whitford, 1989). One concern is that it may alter bone mechanical properties (Mousny *et al.*, 2006), which may present clinically an altered risk of bone fracture. The mechanical properties of bone result from the composition and properties of the bone material as well as the amount and structure of the bone present, all of which can be affected by fluoride. The response of bone to fluoride is complex and dose-dependent, engaging different mechanisms at low, medium, and high doses (Boivin and Meunier, 1990; Grynpas, 1990; Turner *et al.*, 1993; Yan *et al.*, 2007; Mousny *et al.*, 2008). Multi-decade exposure to environmental fluoride (~1 mg/day) therefore cannot be modeled in animals or humans by using higher doses and shorter times. Epidemiological techniques have been used to investigate the association of fracture risks with fluoride exposure at these low levels (Allolio and Lehmann, 1999); the results are generally inconclusive, with the exception of a study which found increased rates of fracture only for very low or very high exposure (Li *et al.*, 2001).

Here, we compare tissue-level data from bone specimens from a fluoridated region (Toronto) *vs.* a non-fluoridated region (Montreal). Compressive mechanical testing of specimens was used as a proxy for fracture risk (Turner and Burr, 1993). We sought to complement existing epidemiological findings by examining bone samples from these populations directly, with the aim of determining if a relationship existed between the physical properties of the bone and the fluoride content. Data for samples from the two regions, as well as bone samples in the highest and lowest quartiles of fluoride content, were also compared. We hypothesized that the direct measurement of bone tissue from individuals residing in municipalities with and without fluoridated water would reveal a relationship between fluoride content and structural or mechanical properties of bone.

## MATERIALS & METHODS

### Specimen Collection

Femoral heads were collected from patients undergoing total hip arthroplasty at two hospitals in Canada, Mount Sinai Hospital in Toronto and the Jewish General Hospital in Montreal, between September 1996 and August 2000.

**Table 1.** Information on Patients and Bone Samples, by Region

	Toronto (fluoridated)	Montreal (non-fluoridated)
Fluoride content (ppm)		
Mean $\pm$ SD	1030 $\pm$ 60*	643 $\pm$ 35*
Range	192–2264	270–1200
Age of donors (yrs)		
Mean $\pm$ SD	66 $\pm$ 11*	70 $\pm$ 13*
Gender		
Male	26	15
Female	27	24
Disease state	47 osteoarthritis 2 osteoporosis 1 rheumatoid arthritis 2 avascular necrosis 1 osteonecrosis	28 osteoarthritis 7 osteoporosis 2 rheumatoid arthritis 1 ankylosing necrosis 1 psoriatic arthritis

Municipal water supplies in Toronto have been fluoridated at 1 ppm for more than four decades; Montreal has never had fluoridated water. The use of human study participants was approved by the institutional review board of the University of Toronto. Informed consent was obtained from all patients.

The femoral heads were stored at  $-70^{\circ}\text{C}$  prior to being tested. A cylinder of bone, approximately 6 mm in diameter and 6 mm long, was excised from the center of each femoral head, cleaned and weighed, and tested in compression (see below), after which its fluoride content was determined by neutron activation analysis (Mernagh *et al.*, 1977). Three blocks of bone were excised from the inferior (non-loaded) surface of each femoral head and embedded in Spurr resin. One block was approximately 15 x 15 x 5 mm and was used for determination of mineralization by backscattered electron imaging. Two smaller cubes (approximately 5 mm/side) were excised from a point near the apex of the head (inferoproximal) and toward the shaft of the femur (inferodistal) and used for microhardness testing. The exposed faces were polished to a 0.01- $\mu\text{m}$  finish.

### Mechanical Testing

The dimensions of each cancellous core were measured with a micrometer (together with the mass, these numbers were used to determine the density), and the sample was then tested in unconfined compression at 1 mm/min in a universal testing machine (Instron 1011 or 4465, Instron Corp., Canton, MA, USA; LabVIEW, National Instruments Corp., Austin, TX, USA) until failure occurred. The compressive modulus, yield stress, ultimate compressive stress, strain at ultimate compressive stress, energy to failure, and energy to yield were determined (Turner and Burr, 1993).

### Microhardness Testing

Microhardness measurements were conducted on the embedded bone samples by means of a hardness tester equipped with a Knoop diamond indenter (HM-122, Mitutoyo, Aurora, IL, USA). Each indentation was made under a load of 25 g with a duration

of 10 sec. Ten indentations were made in the subchondral bone, equally spaced along the width of the specimen. A further 10 indentations were made at random locations in the trabecular bone of the specimen. The Knoop hardness (KH) was calculated from the length of the indentations by software in the test system.

### Backscattered Electron Imaging

We used backscattered electron imaging (Grynopas *et al.*, 1994) to quantify bone mineralization on the embedded bone samples (coronal face). The samples were imaged by scanning electron microscopy (Hitachi S-2500, Nissei Sangyo America Ltd., Mountain View, CA, USA) and a backscattered electron detector (Link Tetra, Oxford Instruments, Abingdon, UK). We analyzed the image of the bone by dividing the grayscale range of the bone image into 'bins' (7 for the cancellous bone and 8 for the subchondral bone) and determined the percentage of the image that was at each level, producing a profile of the mineralization of the bone. A weighted average of the mineralization was then calculated:

$$WA = \sum_{n=1}^{7 \text{ or } 8} nx_n.$$

### Statistical Analysis

Statistical tests (*t* tests and linear regressions) were performed with Sigmapstat (Systat Software Inc., San Jose, CA, USA). We used heteroscedastic or homoscedastic *t* tests, as appropriate, to identify differences between groups. Statistical significance is reported if  $p < 0.05$ .

## RESULTS

### Sample Information

Information about patients and sample characteristics can be found in Table 1. In total, 92 femoral heads were collected: 53 samples from patients residing in the Toronto area (mean age  $\pm$  SD: 66  $\pm$  11 yrs), and the remaining 39 from Montreal residents (70  $\pm$  13 yrs).

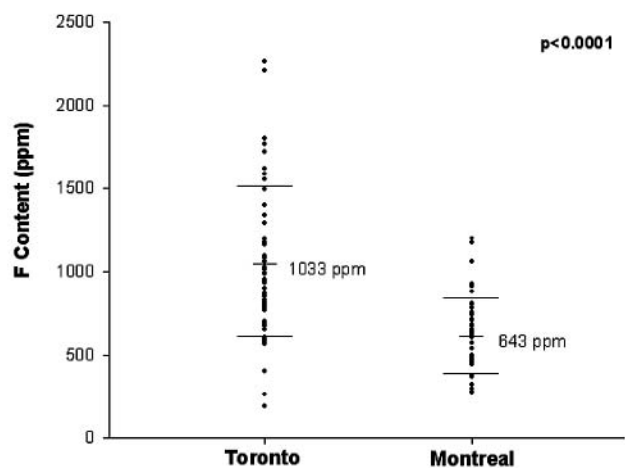
### Fluoridated vs. Non-fluoridated Region

#### Fluoride Content

The fluoride content of bone from individuals residing in Toronto was significantly higher ( $p < 0.0001$ ) than that of those from Montreal. Note, however, that the range for the Toronto bones fully subsumed the range of the Montreal bones (Fig. 1).

#### Compressive Mechanical Properties

The mean density of cancellous cores from the Toronto specimens was significantly greater than that of those from Montreal ( $p < 0.05$ ). However, the density of cancellous cores in this study did not correlate closely with either the fluoride content or the age (data not shown). The mean strain at ultimate compressive stress (UCS) of bone from the Toronto donors was greater than that of their Montreal counterparts, as was the energy absorbed to failure ( $p < 0.05$ ) (Table 2).



**Figure 1.** Fluoride content of bone samples from Toronto and Montreal. The error bars indicate standard deviations. The mean fluoride content of bone samples from Toronto ( $n = 53$ ) residents was higher ( $p < 0.0001$ ) than that of those from Montreal ( $n = 39$ ) residents. Note, however, that the range of fluoride contents in the non-fluoridated region is completely subsumed by those in the fluoridated region.

### Mineralization

No significant differences were observed in the degree of mineralization of the bone between the two regions, by BSE imaging (data not shown). At the inferoproximal (apex) site, the microhardness of the subchondral and of the cancellous bone was greater for specimens from Toronto than from Montreal (subchondral KH,  $43.7 \pm 1.1$  vs.  $38.8 \pm 1.5$ ; cancellous KH,  $44.3 \pm 1.1$  vs.  $39.8 \pm 0.9$ ;  $p < 0.05$ ). No differences were observed at the inferodistal site.

## Comparison of Properties by Fluoride Content

### Description of Quartiles

We used the fluoride content to identify bone samples in the top and bottom quartiles (23 samples each), with mean fluoride contents of  $1434 \pm 70$  and  $449 \pm 25$  ppm, respectively (ranges: 1082–2264 and 192–582 ppm). In the top quartile, 21/23 samples were from the fluoridated region. However, more than a quarter (6/23) of the samples in the bottom quartile were *also* from the fluoridated region. The patients in the top quartile were older than those in the bottom ( $70 \pm 11$  vs.  $62 \pm 14$  yrs of age;  $p < 0.05$ ), consistent with an increase in fluoride accumulation with age (Richards *et al.*, 1994; Chachra, 2001).

### Compressive Mechanical Properties

In contrast to the comparisons by city, the density of the cancellous core was unchanged between the quartiles. Despite this, the yield stress and the ultimate compressive stress were greater for the bottom quartile than for the top quartile (yield stress,  $5.4 \pm 0.8$  vs.  $7.5 \pm 0.6$  MPa; UCS,  $6.0 \pm 0.9$  MPa vs.  $8.4 \pm 0.6$  MPa). No other differences were observed between the quartiles.

**Table 2.** Mechanical Properties of Bone Samples, by Region (mean  $\pm$  SEM; \* $p < 0.05$ )

	Toronto (fluoridated)	Montreal (non-fluoridated)
Density ( $\text{g}/\text{cm}^3$ )	$0.90 \pm 0.04^*$	$0.75 \pm 0.05^*$
Compressive modulus (MPa)	$266 \pm 21$	$232 \pm 21$
Yield stress (MPa)	$7.3 \pm 0.6$	$6.6 \pm 0.5$
Energy to yield ( $\text{MJ}/\text{m}^3$ )	$0.14 \pm 0.02$	$0.14 \pm 0.02$
Ultimate compressive stress (MPa)	$8.3 \pm 0.7$	$7.3 \pm 0.6$
Energy to failure ( $\text{MJ}/\text{m}^3$ )	$0.33 \pm 0.06^*$	$0.21 \pm 0.02^*$
Strain at ultimate compressive stress (%)	$7.9 \pm 0.3^*$	$6.9 \pm 0.3^*$

The mean density of the cancellous cores was greater for the Toronto ( $n = 53$ ) specimens than for the Montreal ( $n = 39$ ) specimens. In compression, the strain at failure and the energy absorbed to failure were significantly increased in the Toronto specimens compared with their Montreal counterparts. The microhardness values of both the subchondral and cancellous regions of bone were also greater for the Toronto samples compared with the Montreal samples.

### Mineralization

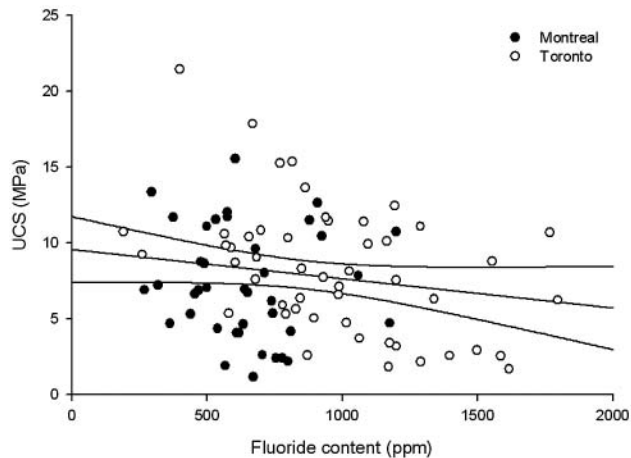
There was no difference in the degree of mineralization between the two quartiles, as measured by BSE imaging (data not shown). However, the top quartile had consistently higher microhardness than the bottom quartile, and significant differences were observed for two of the four sites: subchondral bone at the inferoproximal site (KH  $45.4 \pm 1.7$  vs.  $36.9 \pm 0.2$ ;  $p < 0.05$ ) and cancellous bone at the inferodistal site (KH  $41.9 \pm 0.5$  vs.  $38.4 \pm 0.8$ ;  $p < 0.05$ ).

## Variability in the Data

A plot of the ultimate compressive stress as a function of fluoride content suggests that there is a weak negative relationship between them (Fig. 2). Note the variability, however: The fluoride concentration accounts for less than 5% of the scatter in the data. In addition, the fluoride content increases with age, and the ultimate compressive stress decreases with age, which further suggests that any relationship between ultimate compressive stress and fluoride may be an artifact of these other relationships (Chachra, 2001).

## DISCUSSION

Epidemiological studies have failed to observe an effect of municipally fluoridated drinking water on bone (McDonagh *et al.*, 2000), but the safety of long-term water fluoridation remains uncertain in public discussions. In this study, we measured the physical properties and fluoride content of the bone samples directly. We then assessed the effect of water fluoridation in three different ways: (i) a comparison of samples from residents of municipalities with fluoridated (Toronto) and non-fluoridated (Montreal) water (this is analogous to a retrospective cross-sectional epidemiological study); (ii) a comparison of bone



**Figure 2.** Relationship between ultimate compressive stress and fluoride content. The ultimate compressive stress, as well as the yield stress, declined with increasing fluoride content of the cancellous core ( $n = 92$ ;  $R^2 = 0.048$ ,  $p < 0.05$ ). However, less than 5% of the variation can be attributed to the fluoride concentration. As well, the fluoride content also increases with age (see Appendix), so this observed decline is likely to be at least partially attributable to increasing age.

samples from the upper and lower quartiles of fluoride content; and (iii) a comparison of the physical properties with the fluoride content itself, treated as an independent and continuous variable.

A striking finding of this study was the lack of a strong relationship between fluoride exposure and bone fluoride content. This manifested as the wide range of bone fluoride content in the specimens from the fluoridated municipality which, in turn, entirely subsumed the observed range for samples from the non-fluoridated municipality. This approach of comparing samples (or, in the case of epidemiological studies, the fracture rates) from two cities may therefore not be able to differentiate between two populations on the basis of fluoride exposure, whether a result of different patient histories (residency, diet) or due to the wide variability in responses to fluoride ingestion (see below). These ambiguous findings from the comparison of municipalities suggested the more direct approach of comparing the upper and lower quartiles by fluoride content.

The differences observed between quartiles are in contrast to the differences observed between cities. Between quartiles, the density is unaltered, but the strength of the bone is lower for the more fluoridated group, which is consistent with some previous animal studies (Mousny *et al.*, 2006). Between cities, the density is greater for the bones from the region with municipal fluoridation, but the strength of the bone is unchanged, while the strain at the ultimate compressive stress (UCS) and the energy absorbed to failure are greater. Because the energy absorbed to yield was identical in the two groups, this suggests that the difference in energy absorption is a consequence of the post-yield behavior; the greater strain at UCS from the fluoridated samples

results in a greater energy absorption to failure, which means that these bones may be more ductile and tough. This may be a consequence of an effect of fluoride on the interface between the mineral and organic phases (Kindt *et al.*, 2008; Mousny *et al.*, 2008; Thurner *et al.*, 2009).

Most importantly, the extremely wide variability in properties makes it difficult to point definitively to a fluoride-related effect. The data presented here show a wide variation in fluoride content, mineralization, structure, and mechanical properties. Fluoride incorporation into bone depends on many factors, including ingestion from sources in addition to water (Burt, 1992), age, duration of residency (Richards *et al.*, 1994), renal function and other disease states (Ekstrand and Spak, 1990), remodeling rate (Ishiguro *et al.*, 1993), and genetic susceptibility (Dequeker and Declerck, 1993; Mousny *et al.*, 2006). About 40% of the population in areas with water supplies naturally fluoridated at very high levels are unaffected by skeletal fluorosis (Choubisa, 2001), and about a third of patients who receive fluoride as a therapy for osteoporosis are described as 'non-responders' (Dequeker and Declerck, 1993), indicating that intrinsic susceptibility to fluoride varies with the individual. A genetic basis for these differences is supported by research with different strains of mice (Mousny *et al.*, 2006, 2008). In a large, diverse urban center like Toronto, therefore, one would expect that the population would display a range of genetic susceptibilities to fluoride, which may in turn explain the broad range in fluoride content measured for Toronto specimens. This may also be part of the explanation for the contrasting pattern of differences between cities and quartiles.

Because the bone samples for this study were obtained from patients undergoing surgery, the patients were generally older; it is possible that they were not representative of the larger population. However, aged populations are likely to be the most vulnerable to any negative effects of municipal fluoride administration because of both fluoride accumulation in bone over time (Richards *et al.*, 1994) and age-related declines in the mechanical properties of bone (Mosekilde and Danielsen, 1987).

Many decades of epidemiological studies have shown minimal evidence of any effects of fluoride administration on bone, and it is therefore very unlikely that municipally fluoridated water affects adults with healthy bone. In this study, no effects of fluoride on mineralization (by BSE) and no substantive negative effects of fluoride administration on bone mechanical properties were observed. Our analysis of samples at the tissue level, rather than the population level, reveals high levels of variability in response to water fluoridation, which may account for the lack of differences observed in epidemiological studies (McDonagh *et al.*, 2000). While we cannot definitively rule out an effect of low-level fluoride accumulation over long periods of time, especially if specific individuals have a genetic or disease background that renders them unusually susceptible to fluoride, it nevertheless appears that the contributors to bone health are too many and varied, and any possible effect of municipal fluoride ingestion is too small, for municipal water fluoridation to be a significant determinant of bone health within the general public.

## ACKNOWLEDGMENTS

This work was funded by a grant from the Canadian Institutes of Health Research. The authors acknowledge the technical assistance of Adeline Ng, Maria Mendes, and Douglas Holmyard, and thank the participating surgeons: Drs. Carol Hutchison and Allan E. Gross at Mount Sinai Hospital, and Drs. David Zukor and Olga Huk at the Jewish General Hospital. This paper is based on a thesis submitted to the School of Graduate Studies, University of Toronto, in partial fulfillment of the requirements for the PhD degree of Debbie Chachra.

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